Morphogenesis: how can periodic patterns be generated from non-linear interactions?

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Abstract: The phenomenon of morphogenesis can be explained by a mechanism containing diffusion-driven instabilities, proposed by Alan Turing. Morphogenesis is about the formation of anatomical structures and periodic patterns in living organisms that can be found in nature. Here I focused on the role of diffusion in the emergence of stationary periodic structures from small perturbations of the homogeneous state. Specially, which conditions parameter values must accomplish to drive pattern formation. The investigation is centered on the non-linear interactions in a system of two different chemical species and which significative changes in pattern arising are done if we add a binding substrate to this kind of system, in concrete, how does it change instability conditions. The study is done through analytical and computational methods to find these conditions and to verify them with simulations.

I. INTRODUCTION

How does the self-organization of identical cells in biological systems behave in order to form spatial periodic structures of different cell types? We can obtain a heterogeneous pattern from a homogeneous state by adding diffusion and fulfilling some necessary conditions, which are analyzed here. In other words, diffusion breaks the initial symmetry of a homogeneous system compound by at least two chemical substances that interact non-linearly through reaction-diffusive mechanisms [1]. This was a revolutionary idea, proposed by Alan Turing, since the common sense makes us think that diffusion tends to homogenize any difference, instead of leading to spatial periodic structures in the stationary state.

The study presented here brings the attention on which are the requirements that diffusion coefficients must satisfy for pattern formation to arise. Since another important aspect involved is the removal rate of a chemical substance, I also want to study its connection in the reaction-diffusion mechanism. Therefore, the study considers which factors drive the instability of the homogeneous, uniform, state.

Here I take as a description of real systems model equations that are non-linear and spatio-temporal partial differential. They are much easier to study both analytically and numerically than the real system. Therefore, the study of diffusion and removal rate influence on pattern generation will be done in two ways: by linear stability analysis and numerical simulations.

Firstly, I will study the simply case of one diffusive molecule in one dimension that is produced and degraded locally, which leads to gradients in this molecule spatial distribution. This will help us to understand better how diffusion and removal rate mechanisms work and is a very relevant point to the morphogenesis as it is mentioned in articles [1,2].

Then, I will focus on an activator-inhibitor system, that is a simple model compounded by two different chemical species. One of them is called activator since it triggers the production of the other specie called inhibitor, that slows down activator's creation, as it is explained in article [3]. Here I want to figure out how these parameters affect on pattern formation in a simple model and what are the particular conditions for this situation by developing the linear stability analysis as in article [3].

As it is explained by A. Turing in article [1] and by A.J. Koch and H. Meinhardt [3], a necessary condition for pattern formation is the presence of a notable difference between activator's and inhibitor's diffusion. But it is still unclear if activator and inhibitor may have such a big difference in their diffusion parameters in real systems. In fact, many articles sustain the opposite, i.e. they usually have similar diffusion coefficients [4,5]. Therefore, it was proposed that the union of a binding substrate to the activator of the mentioned system may lead to Turing patterns event though the diffusion coefficients are close to each other, since this binding modifies activator's effective transport [4,5].

So finally, I will analyze a much more complex system compound by three elements, which means to add a binding substrate to the activator-inhibitor system. In this context, how does it affect Turing conditions for pattern arising? But what happens if these molecules have the same diffusion coefficient? Can we obtain Turing patterns? And how does the removal rate influence on periodical structure formation? Here I took as reference guide articles [4,5].

II. RESULTS

A. Gradient of a chemical specie

To study the distribution of a diffusive chemical specie concentration h in the steady state in one dimension I describe its behavior as a space-temporal equation of partial derivatives:

$$\frac{\partial h}{\partial t} = \mathrm{D}\frac{\partial^2 h}{\partial x^2} - \mu h, \qquad (1)$$

where $h(x=0) = A$. The above dynamics only considers diffusion, with coefficient D, and degradation, with rate μ. The boundary condition sets a constant concentration, and acts as a local source.

This equation allows us to study quantitatively which will be the repercussion of diffusion and removal rate on inhibitor's spatial gradient. First, I solve analytically Eq. (1) in an infinite space in the stationary state. The chemical substance has an exponential behavior in the stationary state:

$$h(x) = Aexp\left(-\sqrt{\frac{\mu}{D}}x\right) = Aexp(-\frac{x}{\lambda}) \qquad (2)$$

with characteristical length: $\lambda = \sqrt{\frac{D}{\mu}}$. Applying logarithms to both sides, a lineal relation with $\sqrt{\mu/D}$ dependence will appear, that is inversely proportional to the characteristical length. This way I can study the consequences of diffusion and removal rate on concentration distribution.

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The analysis can also be done by numerical integration of equation (1) taking a time and -space discretization. Here I discuss four different cases choosing different diffusion and removal rates values and calculating concentration for each space point in the steady state by numerical simulations. Since I am studying numerically a case with finite length, there will show up effects of periodic boundary conditions (Fig.1). In an infinite length case as I go further from the initial point (where the concentration is fixed) the inhibitor distribution will decrease exponentially to zero and will stabilize there. But in this system (with finite length) what we can observe is that the concentration reduces its value until a minimum, which is lower as μ/D coefficient increases) and it is followed by an exponential growth without reaching concentration 1 at the final point.

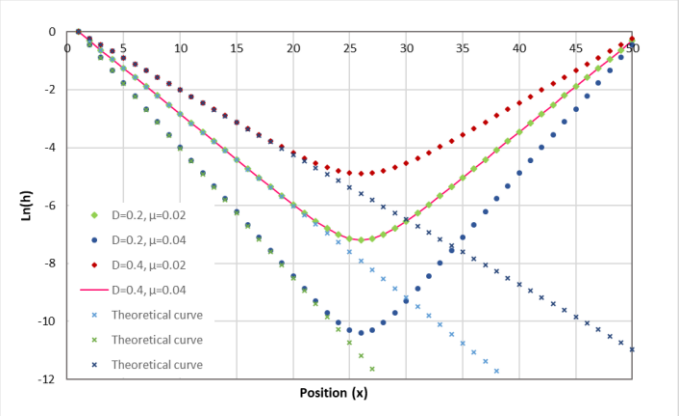

FIG. 1: Logarithm of inhibitor's concentration as position function in one dimension for a lattice of length L=50 comparing with theoretical curves, which are obtained from equation (2) and adding the parameters appointed in the graphic. Two of the cases studied, $(D = 0.2, \mu = 0.02)$ and $(D = 0.4, \mu = 0.04)$, correspond to the same characteristic length $\lambda = \sqrt{10}$.

In conclusion, the concentration decays in space exponentially, stronger as bigger is the removal rate and smaller the diffusion. In particular, the range will be shorter, and it will concentrate at focus surroundings, where we keep the concentration constant, as we can appreciate in figure 2. If we take D and μ realistic values (as appears in article [3]), that are $D \sim \frac{\mu m^2}{s}$ and $\mu \sim \frac{1}{min}$, therefore the characteristical length in a real system will be $\lambda = \sqrt{\frac{D}{\mu}} = 7{,}75 \mu m$.

B. Activator - Inhibitor system

Considering that a non-linear interaction of two chemical diffusive substances can create periodic spatial structures, I put emphasis on an analysis of an activator-inhibitor system:

$$\frac{\partial a}{\partial t} = \rho_a \frac{a^2}{(1+\kappa_a a^2)h} - \mu_a a + \sigma_a + D_a \nabla^2 a \quad (3a)$$

$$\frac{\partial h}{\partial t} = \rho_h a^2 - \mu_h h + \sigma_h + D_h \nabla^2 a, \quad (3b)$$

which has been previously proposed and studied by linear stability analysis and numerical simulations [1]. Here a is the concentration of the activator and h inhibitor's concentration; D_x is the diffusion coefficient, ρ_x is cross-reaction coefficient, μ_x is the removal rate and σ_x is the basic production term, where x corresponds to a or h. κ_a is a saturation constant.

i. Linear stability analysis

For a general model of a system of two chemical species:

$$\frac{\partial a}{\partial t} = f(a,h) + D_a \nabla^2 a, \quad (4a)$$

$$\frac{\partial h}{\partial t} = g(a,h) + D_h \nabla^2 h, \quad (4b)$$

with the homogeneous steady-state solution (a_o, h_o) which are constants that verify

$f(a_o, h_o) = 0\,;\; g(a_o, h_o) = 0\,.$

Now we apply a small perturbation to the homogeneous state

$a(\vec{r},t) = a_o + \delta a(\vec{r},t);\; h(\vec{r},t) = h_o + \delta h(\vec{r},t).$

Then, the equations will be:

$$\frac{\partial (a_o + \delta a)}{\partial t} = f(a_o + \delta a, h_o + \delta h) + D_a \nabla^2 a_o + D_a \nabla^2 \delta a.$$

Since I picked small fluctuations I can obtain linearized equations, considering up to first order terms

$$f(a_o + \delta a, h_o + \delta h) \simeq f(a_o, h_o) + \frac{\partial f}{\partial a}|_{a_o,h_o} \delta a + \frac{\partial f}{\partial h}|_{a_o,h_o} \delta h.$$

Accordingly, the linearized equation for the activator results

$$\frac{\partial (\delta a)}{\partial t} = \frac{\partial f}{\partial a}|_{a_o,h_o} \delta a + \frac{\partial f}{\partial h}|_{a_o,h_o} \delta h + D_a \nabla^2 \delta a.$$

Repeating the same process for the inhibitor, the new dynamics will have the following solutions:

$\delta a(\vec{r},t) = \delta a_o e^{\omega t} cos(k\vec{r}),\; \delta h(\vec{r},t) = \delta h_o e^{\omega t} cos(k\vec{r})$

where $k_n = \frac{2n\pi}{L}\,; n = 0, 1, 2 \ldots, L-1$ due to periodic boundary conditions. Therefore, taking the following notation:

$$\frac{\partial f}{\partial a}|_{a_o,h_o} = f_a|_{(a_o,h_o)}$$

I obtained:

$$L \begin{bmatrix} \delta a \\ \delta h \end{bmatrix} = 0$$

$$L = \begin{pmatrix} f_a|_{(a_o,h_o)} - \omega_n - D_a k_n^2 & f_h|_{(a_o,h_o)} \\ g_a|_{(a_o,h_o)} & g_h|_{(a_o,h_o)} - \omega_n - D_h k_n^2 \end{pmatrix}$$

which can be re-written as

$$L = J - \omega_n I - k_n^2 \begin{pmatrix} D_a & 0 \\ 0 & D_h \end{pmatrix}$$

being J the Jacobian of the system without diffusion. Since I want non-trivial solutions, the solutions are obtained from:

$$\det L = 0 \rightarrow \omega_n^2 + \alpha \omega_n + \beta = 0$$

where

$\alpha = k_n^2 (D_a + D_h) - (f_a + g_h);$

$\beta = D_a D_h k_n^4 - (f_a D_h + g_h D_a) k_n^2 + det\, J \quad (5)$

and a one-dimensional system has been considered.

Since we have a second-degree equation, the solutions for the frequency are: $\omega_n = \frac{-\alpha \pm \sqrt{\alpha^2 - 4\beta}}{2}$ [3,6].

From this expression we can conclude that $\omega_n \in Re\; \alpha^2 \geq 4\beta$ and $\omega_n \in C\; \alpha^2 < 4\beta$.

The representation of the dispersion relation $\omega_n(k_n)$ is an analysis that can provide us with information about which homogeneous state of the system is linearly unstable to small fluctuations. Therefore, whether the real part of eigenfunctions are negative or positive, the perturbation of the homogeneous stationary solution will grow or will decay exponentially with time. So, if $Re(\omega_n) < 0$ our homogeneous state of the system will be stable.

Turing conditions for pattern arising require the homogeneous state of the system to be stable without diffusion and unstable to small perturbations when diffusion

is incorporated. In equations (5) the role of diffusion can be appreciated as it modifies ω_n value and can make it positive or negative.

In diffusion absence, which corresponds to $k_n = o$, we see that in order to have $Re(\omega_n) < 0$ we must impose $\alpha > 0$, which gives us the first condition. To get $Re(\omega_n) > 0$, which is the condition that has to be accomplished in diffusion presence in order to get Turing patterns, α must be negative or β negative from the equation for frequency solution. But the homogeneous solution has to be stable without diffusion, which leaves us the second condition $\beta < 0$. Since Turing patterns are generated if the homogeneous state is stable to small fluctuations in diffusion absence and unstable in its presence, therefore, diffusion is necessary for spatial patterning.

Since I want to derive the conditions for spatial pattern generation in a system composed by two different chemical species subjected to reaction diffusion mechanisms of the model activator-inhibitor form (3), I do the corresponding adimensionalization, for calculation convenience, that is:

$$\bar{t} = \mu_a t,\ \bar{l} = \sqrt{(\frac{\mu_a}{D_h})} l,$$

$$\bar{a} = \frac{\mu_a \rho_h}{\mu_h \rho_a} a,\ \bar{h} = \frac{\mu_a^2 \rho_h}{\mu_h \rho_a^2} h.$$

and I get next expressions (to simplify the notation I dropped the overbars) [1]:

$$\frac{\partial a}{\partial t} = \frac{a^2}{h} - a + D\nabla^2 a \qquad (6a)$$

$$\frac{\partial h}{\partial t} = \mu(a^2 - h) + \nabla^2 a \qquad (6b), \text{ where } D = \frac{D_a}{D_h} \text{ and } \mu = \frac{\mu_h}{\mu_a}$$

Hence, since $f_a = \frac{2}{a_o} - 1$, $f_h = -\frac{1}{a_o^2}$, $g_a = 2\mu a_o$ and $g_h = -\mu$ equations (5) for this case will be:

$$\alpha = (1 + D)\kappa_n^2 + \mu + 1 - \frac{2}{a_o};$$

$$\beta = D\kappa_n^4 + \left(\mu D + 1 - \frac{2}{a_o}\right)\kappa_n^2 + \mu \qquad (7)$$

where $a_o = 1$ and $h_o = a_o^2$.

So, for the case without diffusion α and β from (7) will present the following expression:

$$\alpha = \mu + 1 - \frac{2}{a_o};\ \beta = \mu \qquad (8)$$

Since $Re(\omega_n) < 0$, α must be positive and I obtain the first stability condition

$$\mu + 1 - \frac{2}{a_o} > 0\ . \qquad (9)$$

When we add diffusion, we go back to expressions (7). Considering the equation of dispersion relation, we see that if we want spatial patterns to arise we demand $Re(\omega_n) > 0$, we need the first condition (9) to be fulfilled and moreover $\beta < 0$, which leads us to the second condition

$$\mu D + 1 - \frac{2}{a_o} < 0 \text{ and } \left(\mu D + 1 - \frac{2}{a_o}\right)^2 - 4D\mu > 0. \qquad (10)$$

From equations (9) and (10) it can be deduced that $D < \mu$, and since $\mu > 1 \rightarrow D < 1$, which means that the activator must diffuse slower than inhibitor. In consequence, spatial pattern will arise if diffusion and removal rate will satisfy these expressions (9) and (10).

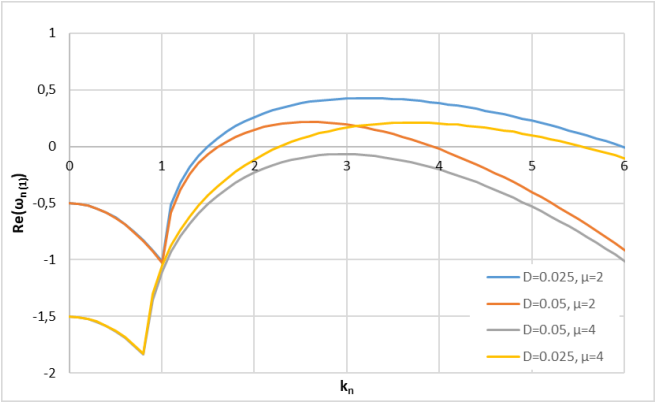

FIG.3: Representation of the dispersion relation $\omega_n(k_n)$ for four different cases.

For cases $D = 0.025, \mu = 2$; $D = 0.025, \mu = 4$ and $D = 0.05, \mu = 2$ from Figure 3 we can conclude that in diffusion absence (it is the same as $\kappa_n = 0$) the system will be stable, but in diffusion presence the system becomes unstable to perturbations and we can obtain spatial patterns. Furthermore, the possibility of getting periodic structures is enhanced when diffusion decreases, since the interval of instable modes enlarges. A smaller removal rate also can help because it moves up the curve, getting a bigger $Re(\omega_n)$.

In the other case ($D = 0.05, \mu = 4$), the system is stable in both situations, in diffusion absence and presence because $Re(\omega_n) < 0$. Hence, no pattern arising will happen in a system with these parameters.

ii. Simulations

By integrating the full dynamics of the activator-inhibitor system, we can obtain numerical results. Comparing their representation with the linear stability analysis allows us to improve our understanding of the role of diffusion and removal rate in pattern formation. So, here I present the numerical integration results of equations (3) for the same cases studied in the linear stability analysis. The computational program is based on the Euler method of numerical integration in one and two dimensions of a lattice with side length L=50, a time step of integration and spatial step equal to 1. For this purpose, I approached the Laplacian in two dimensions and the first order derivate with the Euler method, respectively [1]:

$$\nabla^2 a(x_{ij}, t) = \frac{a(x_{i+1,j},t) + a(x_{i-1,j},t) + a(x_{i,j+1},t) + a(x_{i,j-1},t) - 4(x_{ij},t)}{\delta x^2}$$

$$\frac{\partial}{\partial t} a(x, t_k) = \frac{a(x,t_{k+1}) - a(x,t_k)}{\delta t}$$

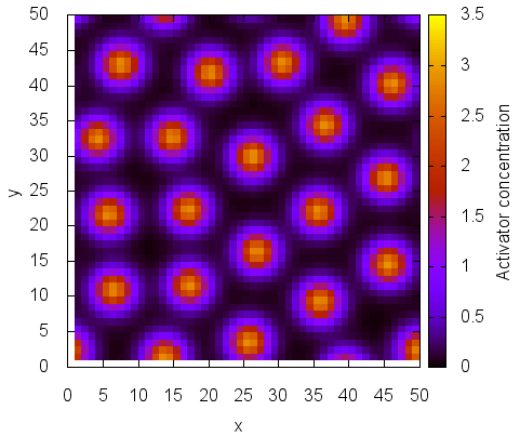

FIG.4: Numerical results for the system with $D = 0.05, \mu = 2$. It is a two-dimensional representation of activator concentration in each point of the square lattice, in the steady state. As brighter is the color as greater is the concentration in that point.

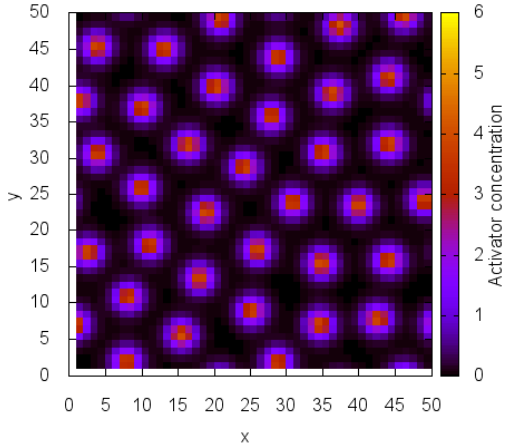

FIG.5: Numerical results for the system with $D = 0.025, \mu = 2$. It is also a two-dimensional representation of activator concentration in each point of the square lattice, in the steady state.

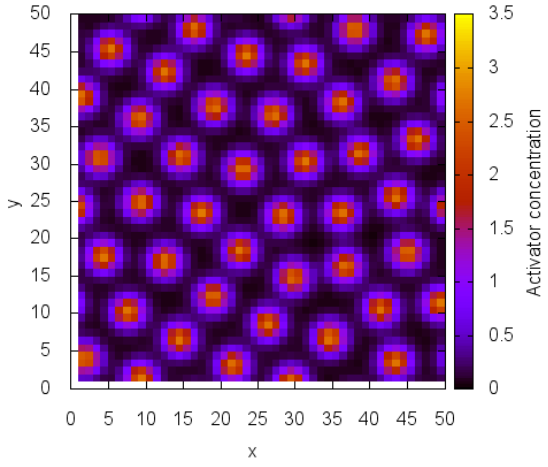

FIG.6: Numerical results for the system with $D = 0.025, \mu = 4$. It is also a two-dimensional representation of activator concentration in each point of the square lattice, in the steady state.

As we can conclude from figure 4,5 and 6 when removal rate is lower and diffusion is bigger, the radius of the activator peaks grows. These parameters have opposite effects because a bigger D represents that activator diffuses faster than inhibitor and a smaller μ indicates that inhibitor disappears quickly. In cases with a smaller diffusion coefficient, peaks radius also goes down. But if diffusion ratio is bigger, activator peaks radius enlarges.

Numerical results for the system with $D = 0.05, \mu = 4$ indicates us that no pattern will be generated, the same as linear stability analysis showed us.

iii. Critical length

The critical length is defined by $Re(\omega_n) = 0$ and we choose ω_n to be real, which involves that α must be zero [1]. Therefore β must be zero too:

$$\beta = D\kappa_n^4 + \left(\mu D + 1 - \frac{2}{a_o}\right)\kappa_n^2 + \mu = 0$$

$$\kappa^2 = \frac{-\left(1+\mu D-\frac{2}{a_o}\right) \pm \left[\left(1+\mu D-\frac{2}{a_o}\right)^2 - 4D\mu\right]^{1/2}}{2D}$$

Given that $k_n = \frac{2n\pi}{L}$, the critical length will be

$$L_c(n) = 2n\pi\sqrt{2D}\left\{\left(\frac{2}{a_o} - 1 - \mu D\right) + \left[\left(\mu D + 1 - \frac{2}{a_o}\right)^2 - 4\mu D\right]^{\frac{1}{2}}\right\}^{-1/2}$$

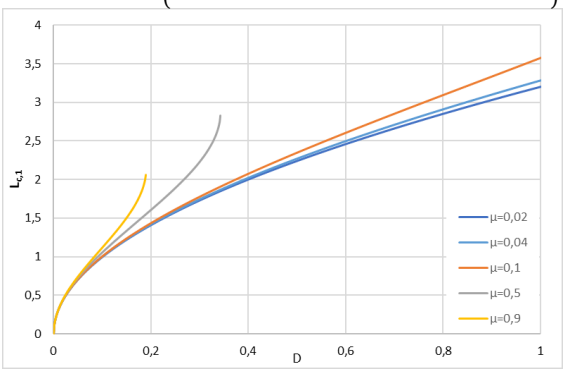

FIG. 7: Critical length as function of the diffusion ratio D, for five different removal rates coefficients, for n=1.

This is an additional interpretation for instability conditions, in some cases (when the homogeneous state is unstable) if a system has a critical length, the system must have a length bigger than this critical length in order to a fluctuation to grow and to obtain a heterogeneous state from a homogeneous one. If an organism is smaller than this length, perturbations applied to the homogeneous state will not develop to give periodic patterns.

iv. Binding substrate

The cases that I listed above show us that the main condition for diffusion-driven-instability with two chemical substances is to have different diffusion coefficients, in particular, inhibitor must diffuse faster than activator. As equation Einstein-Stokes indicates in real systems we find molecules which diffuse at similar rates because they have similar radius: $D\gamma = \kappa_B T$, where $\gamma = 6\pi\eta R$ and R is the radius of a spherical molecule. Given that the radius of different biochemical molecules is expected to be within the same order of magnitude, they will present similar diffusion coefficients. So, it will be problematic to find Turing structures due to $D \approx 1$ [4,5].

There are evidences that show that adding an immobile substrate to a system with two chemical species diffusing at same speed may lead to pattern generation. [4,5] Here I will discuss two cases: whether the immobile substrate binding to activator is reversible or not.

In first place, equations for reversible binding will be; in one dimension [3]:

$$\frac{\partial a}{\partial t} = f(a,h) - k_+ s_o a + k_- sa + D_a \frac{\partial^2 a}{\partial z^2} \quad (14a)$$

$$\frac{\partial h}{\partial t} = g(a,h) + D_h \frac{\partial^2 h}{\partial z^2} \quad (14b)$$

$$\frac{\partial sa}{\partial t} = k_+ s_o a - k_- sa. \quad (14c)$$

Putting together (14a) and (14c), in stationary state:

$$\frac{\partial (a+sa)}{\partial t} = (1+K')\frac{\partial a}{\partial t} = f(a,h) + D_a \frac{\partial^2 a}{\partial z^2},$$

where $K' = K s_o = \frac{k_+}{k_-} s_o$ is the dissociation constant of the complex activator-substrate. So, equations (14) will be:

$$\frac{\partial a}{\partial t} = F(a,h) + D'_a \frac{\partial^2 a}{\partial z^2}, \quad (15a)$$

where $F(a,h) = \frac{f(a,h)}{1+K'}$ and $D'_a = \frac{D_a}{1+K'}$

$$\frac{\partial h}{\partial t} = g(a,h) + D_h \frac{\partial^2 h}{\partial z^2}. \quad (15b)$$

Comparing equations (15) to equations (3) we can find similarities to an activator-inhibitor system and keep the conclusions that we find earlier. So, considering $1 + K' < 1$, which means complex formation of an immobile substrate with activator molecule, we see that we may obtain Turing patterns even though diffusion ratio is close to 1. So, we need a bigger removal rate and smaller diffusion. From figure 3 we see that this hypothesis is not verified since we do not observe an improvement of pattern generation if we switch from orange curve to the yellow one (where μ increases and D decreases).

For irreversible binding [3]:

$$\frac{\partial a}{\partial t} = f(a,h) - k_+ s_o a + D_a \frac{\partial^2 a}{\partial z^2} \quad (16a)$$

$$\frac{\partial h}{\partial t} = g(a,h) + D_h \frac{\partial^2 h}{\partial z^2} \quad (16b)$$

$$\frac{\partial as}{\partial t} = k_+ s_o a. \quad (16c)$$

Relating equations (16a) with (3a), we can see that they are similar except from an increasing of the removal rate. So, a growth of activator's removal rate leads us to a facilitation of getting periodic structures. This is proved by looking at figure 3 and considering a decreasing of μ (since this coefficient is inversely proportional to activator's removal rate): from blue curve to the grey one there is a removal rate decreasing and we see how ω_n passes from negative value to positive allowing fluctuation growing in order to pattern arise.

Therefore, an irreversible binding for the activator-inhibitor system studied here makes easier to obtain Turing patterns from a homogeneous state.

III. CONCLUSIONS

The phenomenology of periodic structure generation can be understood by a simplified model that describes a reaction-diffusion mechanism of the interaction between at least two chemical species. In this study, the focus was put on an activator-inhibitor system where the interaction is non-linear and represented by spatial-temporal partial differential equations. The pattern arising is due to the addition of diffusion to the homogeneous state of the system which is perturbed.

Here we determined which are the conditions for diffusion and removal rate of chemical molecules to fulfill if we want a fluctuation to be amplified in a homogeneous state. In particular, we studied the case of activator-inhibitor model. With this mathematical model we lost realism but instead it allowed us to perform a linear stability analysis and a computational method to go deeper in our knowledge of Turing patterns.

It is crucial to be aware that is difficult to find two chemical species that diffuses at different rates due to their morphology and size. Therefore, we studied here what is the influence of a substrate binding to the complex activator-inhibitor, in particular to the activator as it changes its effective transport, otherwise it will not result in a pattern generation. We saw that if the binding is irreversible it enhances the possibilities of Turing pattern generation comparing to the reversible binding.

For future investigations it will be interesting to analyze this kind of binding for other models, for example, activator-substrate model, and see which aspects are improved in periodic structures generation or whether there is another mechanism of Turing stability conditions relaxation. For instance, how non-diffusible factors can influence on pattern forming conditions, because they are based on the interaction of many components. Therefore, the classical Turing interpretation can be enlarged and adapted to a more realistic framework where are plenty of diverse chemical molecules.

Acknowledgments

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